

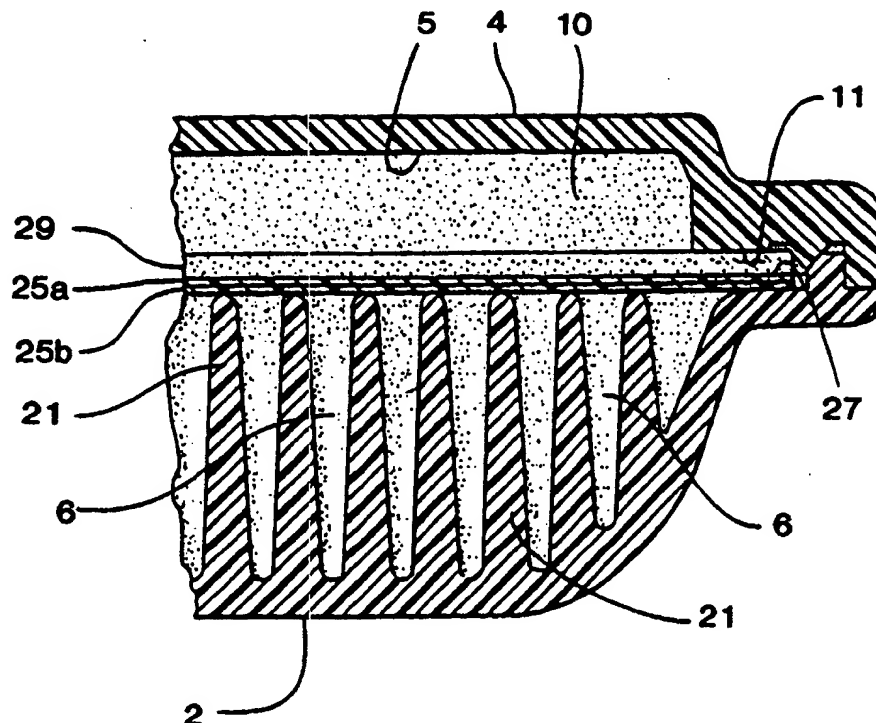
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>A61M 1/36</b>		A1	(11) International Publication Number: <b>WO 98/19722</b>
			(43) International Publication Date: 14 May 1998 (14.05.98)
(21) International Application Number: PCT/US97/19908 (22) International Filing Date: 5 November 1997 (05.11.97)  (30) Priority Data: 08/745,948      8 November 1996 (08.11.96)      US 08/841,163      29 April 1997 (29.04.97)      US  (71) Applicants: PALL CORPORATION [US/US]; 2200 Northern Boulevard, East Hills, NY 11548 (US). BAXTER INTERNATIONAL, INC. [US/US]; Route 120 and Wilson Road, RL, Round Lake, IL 60073-0490 (US).  (72) Inventors: SUTTER, Mark, A.; 7660 Quail Ridge Drive, Dexter, MI 48130 (US). BORTON, Noel, T.; 17860 Dens Drive, Chelsea, MI 48118 (US). BISCHOF, Daniel, F.; 4913 Rain-tree Court, McHenry, IL 60050 (US). CHAPMAN, John; 67 Kevin Avenue, Lake Villa, IL 60046 (US). HERMAN, Robert, E.; 542 Northgate Road, Lindenhurst, IL 60046 (US). SUN, Chong-Son; 530 Golf Lane, Lake Forest, IL 60045 (US).  (74) Agent: JAY, Jeremy, M.; Leydig, Voit & Mayer, Suite 300, 700 13th Street, N.W., Washington, DC 20005 (US).			(81) Designated States: AU, CA, CN, JP, NO, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>

(54) Title: METHOD FOR PURIFYING BLOOD PLASMA AND APPARATUS SUITABLE THEREFOR

## (57) Abstract

A method and device for purifying conventionally treated blood plasma, to remove substantially all residual leukocytes while maintaining useful flow rates for medically useful amounts of plasma, utilize a disposable, heat-sterilizable filter (1) employing one or more depth-type prefilters (29) and at least one intermediate hydrophilic microporous membrane (25a) followed by at least one final hydrophilic microporous membrane (25b) having a smaller pore size than the intermediate membrane within a common housing.



**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## METHOD FOR PURIFYING BLOOD PLASMA AND APPARATUS SUITABLE THEREFOR

### Technical Field

5 The present invention pertains to a method for purifying blood plasma of white blood cells (leukocytes) and undesirable contaminants, particularly viral contaminants, and to an apparatus suitable for this use.

### Background Of The Invention

10 Plasma is the continuous liquid phase of blood which transports the necessary active substance which feed and maintain the body. Included within plasma are electrolytes, soluble sugars and proteins, and numerous enzymes, antigens, etc. While whole blood also contains red and white blood cells (erythrocytes and leukocytes), these components are  
15 substantially removed in the preparation of plasma. Plasma is frequently administered to patients who are seriously ill, for example burn victims. Plasma may also be fractionated to provide fractions enriched in certain components such as Factor VIII to treat many diseases, including Hemophilia.

20 Unfortunately, many disease-causing viruses, such as Hepatitis and HIV, may be transported by plasma if the blood donor has been infected by these diseases. Even without such infection, plasma may be contaminated during collection and subsequent processing. While centrifugation is effective to  
25 remove erythrocytes and the majority of leukocytes, infective viruses cannot generally be removed without resorting to ultracentrifugation. Such treatment is not generally cost effective and moreover may change the chemical make-up of the plasma by separation of larger molecules contained therein.

30 Administration of plasma containing even the most minor amounts of infectious agents can be catastrophic, and thus methods have been proposed to sterilize plasma utilizing chemical sterilizing agents. Unfortunately, if the plasma contains leukocytes which contain or are bound to infectious  
35 agents, these latter may not be destroyed by such processes,

and thus a risk of infection is present. Virtually all leukocytes must therefore be removed.

Simple filtration of the plasma prior to chemical sterilization is problematic, as plasma is unique in the sense that in addition to low molecular weight species and electrolytes which may easily pass through even "tight" membranes, the larger protein species will rapidly form a polarized gel coating on the membrane should the pore size be too small. For example, the albumin fraction of human plasma contains prealbumin and albumin, with molecular weights in the 60,000 to 70,000 range, while fibrinogen, and various immunoglobulins have molecular weights in the range of 300,000 to  $1 \times 10^6$ . The  $\beta$ -lipoproteins, important in the transport of fats and lipids, have molecular weights in the range of  $3 \times 10^6$  to  $20 \times 10^6$ .

When the plasma contains infectious agents such as viruses, the pore size necessary to completely remove these agents, particularly retroviruses, is such that the filter becomes rapidly clogged, thus requiring large filter area or repeated filter replacement. Clogging is particularly important with respect to the small but finite amount of leukocytes present in conventionally prepared plasma. Leukocytes are deformable, and may clog fine pores even though the leukocytes are physically larger than the pore. Further, and as indicated previously, a small pore size may also filter out desirable macromolecules contained in the plasma.

Leukocytes, which are positively charged, have been separated from plasma by membranes having charged sites. The ability to separate leukocytes in this fashion allows membranes with larger pore sizes to be used, as the removal is due to electrostatic attraction rather than physical separation. The larger pore size enhances the useful flow rate. Unfortunately, the charged membranes have a finite number of charged sites which limit their capacity. Moreover, the possibility exists that a given leucocyte may pass through the filter without encountering a charged site to bind it to the filtration medium. Random "pass through" cannot be

tolerated in view of the danger of infection by agents such as hepatitis and HIV.

A suitable filter must not only be capable of removing leukocytes while allowing larger macromolecules to permeate the membrane, but must do so while processing a useful volume of plasma at a useful flow rate and acceptable pressure.

### Summary Of The Invention

The subject invention pertains to a process for purifying blood plasma which has been subjected to centrifugation or filtration to remove erythrocytes and a substantial quantity of leukocytes, wherein all or substantially all the remainder of the residual leukocytes are removed through the use of a sterilizable multicomponent filter stack. The filter stack consists of a prefilter, a leukocyte retaining, intermediate hydrophilic membrane filter ("intermediate membrane") and a final, leukocyte retaining safety hydrophilic membrane filter ("final membrane").

The subject invention further pertains to a steam sterilizable multi-element filter assembly comprising a housing, preferably of sterilizable polymer having inlet and outlet portions, the inlet portion including an inlet port and the outlet portion including an outlet port, the inlet and outlet portions defining a flow channel between the inlet and outlet ports; one or more prefilter(s) retained within the housing fully extending across the flow channel, the prefilter(s) disposed closer to the inlet port than the outlet port; two or more hydrophilic microporous membrane(s) retained within and preferably hermetically sealed to the housing and extending across the flow channel, an intermediate hydrophilic microporous membrane adjacent to the prefilter and disposed closer to the outlet port than the inlet port and at least a final hydrophilic microporous membrane adjacent the intermediate membrane; such that plasma to be purified must pass in order through the prefilter(s), the intermediate hydrophilic microporous membrane, and then the final hydrophilic microporous membrane.

Typically, the purified plasma produced in accordance with the invention is subjected to treatment (e.g., chemical treatment) to inactivate and/or destroy infectious agents such as hepatitis and HIV.

## 5 **Brief Description Of The Drawings**

FIGURE 1 illustrates a side view of an assembled filter of one embodiment of the subject invention;

FIGURE 2a illustrates a side view of the inlet portion of one embodiment of a filter device prior to assembly;

10 FIGURE 2b illustrates a view in plan of the inlet portion of one embodiment of a filter device according to the present invention, from the direction of the sealing surfaces;

FIGURE 3a illustrates a side view of the outlet portion of one embodiment of a filter device prior to assembly;

15 FIGURE 3b illustrates a view in plan of one embodiment of a filter device according to the present invention, from the direction of the sealing surfaces;

FIGURE 4 is a cross-section of the outlet portion of a filter device across section 4-4 of Figure 3b;

20 FIGURE 5 is a detail view of area D of Figures 2 and 3.

## **Specific Description of The Invention**

In the method of purifying plasma according to the subject invention, fresh or frozen plasma is first subjected to customary treatment in which virtually all red blood cells and a substantial portion of white blood cells are removed. Although it is preferable that all leukocytes are removed, this is generally impractical, and it is understood that the plasma to be purified will contain a small but finite number of leukocytes.

30 In operation, the blood plasma flows by gravity, e.g., preferably at a head of from about 15 inches to about 48 inches, more preferably about 28 inches, through the leukocyte filtration device, and from thence into a collection vessel, which may be a collection bag or the like. Attachment of the plasma source (i.e., fresh, frozen, etc.) to the filter may

be made by conventional means. The particular methods of connection, supply, bypass, post-treatment, etc., are not required for an understanding of the method of removing leukocytes from plasma or the device suitable therefor as claimed herein.

Figure 1 illustrates one embodiment of the subject multicomponent filter. Other variations will readily suggest themselves to one skilled in the art. In Figure 1, filter 1 consists of two injection molded portions, an inlet portion 2 and an outlet portion 4. In plan, the device is substantially rectilinear, preferably with rounded corners. However, the shape of the device is not critical, and the device shape as well as filter configuration may be adapted as required for any given application. A flow channel is formed by the interior walls 5 of the outermost surfaces of the device, which rise at a modest angle, e.g., about 5-15°, preferably about 9-10° from the end 7 of the inlet portion most removed from the inlet 9 and the end 8 of the outlet portion most remote from outlet 17. The flow channel of the device comprises the enclosed volume including inlet flow channel 10 and outlet flow channel 6, respectively. The ramps formed by the rising outermost surfaces are terminated by substantially vertical walls 31 and 32 on which inlet 9 and outlet 17 are located.

Figure 2a illustrates a side view of the inlet portion 2 of one embodiment of the subject filter device across section 2a of Figure 2b. At 9 is the inlet through which plasma to be treated flows into inlet flow channel 10. At 11 is a raised annular portion which forms a seal, for example a compression seal, against the filter(s) when the device is assembled. At 13 is a raised annular rib of generally triangular cross-section, which is used to seal the inlet portion 2 of the device to outlet portion 4 (Figures 1, 3a, 3b, 4). Shown at 15 is an optional vent hole which will be plugged or covered by a microporous hydrophobic membrane to allow air to escape the device but not liquid.

Referring to Figure 2b, viewed from the direction of the

sealing surfaces, the perimeter of the inlet portion of the device is substantially planar and is surrounded by a raised annular portion 11 which serves to compress and/or seal the periphery of the prefilter(s). Spaced apart from the raised annular portion 11 is a raised annular rib 13 which serves as a pressure concentrating structure when pressed against the corresponding raised annular mating surface 23 (Figures 3a, 3b, 4) of the outlet portion of the device. Other sealing arrangements will readily suggest themselves to one skilled in the art. At 15 is an optional vent hole which is hermetically sealed to a polytetrafluoroethylene or other microporous venting material, preferably hydrophobic, with a nominal pore size of, for example, 0.02  $\mu\text{m}$ .

Figure 3a is a side view across section 3a of Figure 3b. The outlet is shown at 17. Raised annular mating surface 23 mates with corresponding raised annular rib 13 (Figures 2a, 2b) and is subsequently sealed to the rib 13, preferably by ultrasonic bonding techniques. The outlet channel is shown at 6. The outlet channel is formed by the space enclosed by the interior wall 5 of the outermost surface and the surface of the hydrophilic microporous membranes 25a and 25b (Figure 5), which will be sealed to sealing surface 27.

Referring to Figure 3b, the outlet portion of a device is shown in plan. Preferably, the outlet channel 6 contains a plurality of ribs 21 rising from the interior wall of the outermost surface forming a support member for the filter(s), as shown in section in Figure 4. The ribs generally rise to the plane of the lowermost surface of the filter membrane and provide support to ensure the filter is not deformed, ruptured, or separated from its seal with the device during filtering. Other support members, e.g., screens, perforated plates, and the like may be used as well. If the filter is to be used in low pressure drop applications only, e.g., gravity feed, the support member may be eliminated.

Surrounding the outlet channel is raised annular mating surface 23, which, together with raised annular rib 13 (Figures 2a, 2b), provide a means for hermetically sealing the



device, for example by ultrasonic welding. Prior to assembly of the device, hydrophilic microporous membranes 25a and 25b (Figure 5) are hermetically sealed to annular membrane sealing surface 27, for example by heat sealing. Following sealing of the hydrophilic microporous membranes, prefilter 29 (Figure 5) is placed in position atop the membranes and inlet portion 1 is placed atop outlet portion 2 and the assembly is bonded together, preferably by ultrasonic welding.

Referring to Figure 4, the outlet portion of the device is shown across section 4-4 of Figure 3b. The outlet channel 6 and ribs 21 may be clearly seen. At 26 are the hydrophilic membranes abutting the ribs and the prefilter(s), both shown as a single unit for clarity. The membrane sealing surface 27, interior wall 5, and raised annular mating surface 23 are also shown.

Figure 5 illustrates the placement of the final microporous hydrophilic membrane 25b, intermediate microporous hydrophilic membrane 25a, prefilter 29, and the relative geometries of the various annular surfaces when the device is assembled. The hydrophilic microporous membranes 25a and 25b are sealed, preferably heat sealed, to sealing surface 27, and supported across outlet channel 6 by ribs 21. The prefilter 29 is trapped between the portion of the intermediate hydrophilic microporous membrane atop sealing surface 27 and annular raised portion 11. Raised annular rib 13 has been deformed in the heat sealing process to form a unitary structure with raised annular mating surface 23. The plasma flow is through the inlet 9 into inlet channel 10, through prefilter 29, intermediate membrane 25a, and final membrane 25b into outlet channel 6 and from there to outlet 17 (Figure 1).

In use, the inlet 9 is connected to a plasma supply source, which may supply plasma by gravity flow or under pressure, e.g., through use of a peristaltic-type or plunger-type pump. Air is displaced through optional vent 15 and the plasma passes through the prefilter 29 and then through the hydrophilic microporous membranes 25a and 25b. The prefilter

is a "depth-type" prefilter, and thus the majority of macrocytes and granulocytes and any other large particulates may be trapped by the prefilter without significantly decreasing its flow rate. Of the leukocytes or particulates which pass through the prefilter, the vast majority, virtually, and many times, all of the leukocytes, are prevented from passing into the treatment bag by the intermediate hydrophilic, leukocyte trapping microporous membrane. Any leukocytes, e.g., those of extremely small size which pass through the intermediate membrane, are virtually fully retained by the final membrane, and hence, the plasma passing through the filter will be substantially leukocyte-free, providing the pressure across the filter is not so high as to cause it to separate from the housing or rupture, or to deform the leukocytes to such an extent that they are forced through the fine pores of the final membrane. To ensure that the latter event does not occur, the maximum pore size of the final membrane is selected so as to be considerably smaller than the minimum leukocyte diameter. It is most desirable to remove leukocytes such that their concentration in the plasma is reduced by a factor of at least about  $10^3$ , preferably about  $10^4$ . Most preferably, the final leukocyte concentration is 0.

The prefilter performs the function of removing large particulates and gelatinous substances so as to prevent clogging of the intermediate filter. The prefilter also may remove a substantial portion of large leukocytes, i.e. macrocytes and granulocytes. The intermediate membrane removes the most substantial portion of smaller leukocytes such that plasma exiting the intermediate membrane has had preferably greater than about 90% of leukocytes removed. The final membrane has smaller pores than the intermediate membrane, and ensures that virtually no leukocytes remain in the completely filtered plasma.

With regard to pore size, the pore size and type must be selected with the function of the particular filter element in mind. For the prefilter, for example, a relatively large pore size is selected for rapid filtration yet which will

retain substantially all large particulates. When the prefilter is a membrane-type depth prefilter, for example, the preferred pore size is from about 3  $\mu\text{m}$  to about 10  $\mu\text{m}$ . This relatively large pore size is necessary since the pore size range of membrane filters is ordinarily well controlled. However it is preferred that the prefilter comprise a non-woven depth-type filter. Such filters are available from numerous sources, and may consist, for example, of fiberglass, spun bonded or melt blown polypropylene, polyester, and the like, and may have a nominal, or "average" pore size of from about 0.5  $\mu\text{m}$  to about 5  $\mu\text{m}$ . The smaller average pore size is a reflection of the relatively wide pore size range of such materials and the alternative fluid flow paths which depth-type filters present. The most preferable prefilter is a non-woven fiberglass prefilter available from Hollingsworth & Vose as HB-5341 glass filter medium.

The intermediate membrane filter is a hydrophilic membrane filter which has a pore size range sufficiently small that in combination with the prefilter, greater than about 80% of the leukocytes, more preferably greater than about 90% of the leukocytes, and most preferably in excess of about 95% of the leukocytes, are retained by the combination of prefilter and intermediate membrane. In other words, the intermediate membrane will pass no more than about 20% of the leukocytes, preferably no more than about 10%, and most preferably no more than about 5% of the leukocytes. It is yet more preferable that not more than about 1% of leukocytes pass through the intermediate membrane. The pore size of the membrane may vary somewhat, but is preferably in the range of about 0.9 to about 2.0  $\mu\text{m}$ , more preferably in the range of about 0.9 to about 1.5  $\mu\text{m}$ . The composition of the intermediate membrane is not overly critical, so long as the membrane is hydrophilic. Thus, intrinsically hydrophobic membranes which have been treated to render their surface hydrophilic are suitable, as are also intrinsically hydrophilic membranes. Membranes may be made of, for example, polyacrylates, nylon, polyvinylidene fluoride, polypropylene, polysulfone, polyethersulfone,

cellulose acetate, or nitrocellulose. Charged membranes are suitable as well. Nylon membranes are well suited for use herein. A particularly preferred membrane is SUPOR® 1200, a polyethersulfone microporous membrane with a nominal pore size of 1.2  $\mu\text{m}$  available from Pall Gelman Sciences, Inc., Ann Arbor, Michigan.

The final membrane is selected so as to render the plasma filtrate substantially leukocyte-free. While the intermediate filter might be selected so as to provide leukocyte-free plasma by utilizing a smaller pore size, use of a smaller pore intermediate membrane without a final membrane possesses two distinct disadvantages. First, the smaller pore size would reduce flow shortly after the onset of filtration due to its pores becoming clogged with leukocytes. Second, were leukocytes to pass through the pores, e.g., by the well known distortion of the leukocyte, or by accidental damage affecting the membrane integrity, then the risk of leukocyte-containing plasma, although small, would be significant.

Thus, the final filter is selected so as to provide a pore size small enough to ensure substantially complete leukocyte removal. Since the vast majority of leukocytes and larger particulates and gels have been removed by the prefilter and the intermediate membrane filter, the small pore size of the final membrane will not overly slow the filtration rate. As is the case with the intermediate membrane, the final membrane is hydrophilic, and may be a charged membrane as well. The pore size range of the final membrane is from about 0.3 to about 1.5  $\mu\text{m}$ , and preferably is of a smaller or equal pore size than the intermediate membrane. A pore size range of about 0.4  $\mu\text{m}$  to about 1.0  $\mu\text{m}$  is suitable, and a range of about 0.7  $\mu\text{m}$  to about 1.0  $\mu\text{m}$  is preferred. Particularly suitable is a SUPOR® polyethersulfone microporous membrane with a nominal pore size of 0.8  $\mu\text{m}$ .

The surface area, or effective filtration area (EFA) of the filter may be adjusted according to the volume of plasma to be filtered and the desired flow rate. For example, the subject device has been illustrated with reference to a

planar, substantially rectangular filter capsule. However other shapes are useful as well, including pleated cylindrical filters, spiral wound cylindrical filters, and the like, provided the subject filter stack is used. The internal  
5 volume should be minimized so that retention of plasma by the filter itself is as small as possible. In general, a minimum flow rate of about 1 to about 10 ml/min/cm<sup>2</sup> at 1 psi or thereabouts is desired, more preferably a minimum flow rate of about 2-3 ml/min/cm<sup>2</sup>. Higher flow rates are, of course,  
10 desired. The filter should preferably be capable of filtering a minimum of about 300 ml of human plasma before the flow rate decreases to such an extent that the filter may be considered to be "plugged". The filtration efficiency should be such that a 10<sup>4</sup> reduction of leukocytes from conventionally  
15 prepared plasma containing a normal leukocyte concentration is possible. In the most preferable case, no leukocytes will be present in the filtrate. With the preferred devices according to the present invention, incorporating at least one prefilter and intermediate and final hydrophilic microporous  
20 membranes, suitable filter areas are not particularly limited. For example, cartridge filters with pleated or spiral elements may be designed for large volume filtration, while small units may be provided for filtration of single plasma units. In the latter case, for example, a suitable filter size has an EFA  
25 of from about 15 to about 20 cm<sup>2</sup>. However, units with EFAs of from 0.1 m<sup>2</sup> to several m<sup>2</sup> or larger are also feasible.

The prefilter material should meet USP requirements for particle shedding and Class VI toxicity requirements. Preferably, the material further meets European community  
30 toxicity requirements as well.

Combinations of various prefilters with the same or different pore sizes are also useful. In general, hydrophobic materials such as polypropylene should be treated with a hydrophilizing agent to render the material hydrophilic. Such  
35 agents are known to those skilled in the art. The intermediate and final hydrophilic microporous membranes are preferably ones which can be heat-sealed to the filter housing

by conventional techniques such as ultrasonic bonding. Membranes which may be adhesively bonded or solvent bonded are also acceptable.

As indicated previously, the hydrophilic microporous membranes may be intrinsically hydrophilic or may be hydrophobic membranes surface treated to render them hydrophilic. While the membrane may also include negatively charged sites to aid in leucocyte retention, it is most important that the pore size be such that the pore size alone substantially prevents passage of leukocytes. Hydrophilic microporous membranes of suitable pore size are commercially available, and may be manufactured by processes disclosed in, for example, U.S. Patent Nos. 3,876,738; 4,340,479; 4,473,474; 4,673,504; 4,708,803; 4,711,793; 5,076,935; 4,900,449; 4,964,990; and 5,108,607, which patents are herein incorporated by reference. Preferred hydrophilic microporous membranes are Supor® microporous membranes available from Pall Gelman Sciences.

The housing for the multicomponent filter assembly of the subject invention are preferably prepared from injection molded polymer. The polymer may be a thermoplastic or thermosetting polymer, and should be sterilizable. Moreover, the polymer must not elute toxic metals, oligomers, monomers, or catalysts in the presence of aqueous solutions. Finally, although heat-sealable or solvent-bondable thermoplastics may be used, it is preferable that the polymer be capable of being sealed by ultrasonic or RF welding techniques. Among the suitable polymers are the amorphous polyamides, high temperature polyacrylics and polyesters, and most preferably, the polycarbonates. A preferred polycarbonate is MAKROLON 2658-1112 Natural, available from Miles, Inc.

A series of plasma purifying screening tests were run using conventionally prepared bovine serum and a 28 inch head height. For the purposes of the tests, a 47 mm stainless steel filter holder was utilized to hold the primary membrane and prefilter(s). Unfiltered bovine serum was obtained from American Biologic Tech., Inc.

As a result of these screening tests, it was discovered that the selection of suitable prefilters and membrane filters was not straight-forward. A single membrane of 1.2  $\mu\text{m}$  polyethersulfone plugged virtually immediately. When employed with a prefilter, suitable flow rates could be achieved with "clean" plasma with certain prefilters, however with other plasma samples, plugging occurred rapidly. Some of the many combinations tried exceeded acceptable levels of particle shedding, leaching of heavy metals, or were of high pH. These tests showed that a two-stage (prefilter plus single membrane) is unsuitable, and that a minimally three stage filter stack, including a depth-type prefilter, was necessary.

Testing of plasma (leukocyte-removal) filter devices was performed in a sterile-barrier laminar-flow hood under strict aseptic techniques, with minimal handling of plasma units. Leukocyte-removal filter devices were composed of glass fiber/Supor® 1200/Supor® 800 membranes, with an effective filtration area (EFA) of 17  $\text{cm}^2$ . Human plasma was obtained frozen from the Red Cross, and maintained in a frozen state until the morning of testing. Plasma was thawed using a circulating water bath at constant temperature of 8°C. Plasma from four individual units (ranging in volume from 255 mL to 410 mL) was pooled together in a 3-L sterile collapsible admixture bag, after which testing was performed as soon as possible (less than 30 minutes). Plasma was delivered from the collapsible bag, at 28 inch head height, through a Medical Specialties 103 inch vented administration set.

Four leukocyte-removal filter devices, labeled M1, M2, M3 and M4, were filtered into luer-lock extension sets (total length = 6 inches), which were then fitted to the previously mentioned administration set. (Due to air embolus occluding device M1, only data from the other three devices are reported.) Amounts of plasma delivered through filter devices at time intervals of 10 seconds, 1, 2, 5, 10, 15, 20, 25 and 30 minutes were measured and recorded. Plasma flow was continuous and uninterrupted. After completion of testing, plasma samples were treated as biohazard, then sterilized

(through autoclaving at 121°C for 30 minutes) and properly disposed of. All materials used in testing were likewise sterilized, and disposed of if necessary. Flow data is presented in Table 1.

- 5 While the invention have been described in detail by way of illustration and example, those familiar with the art to which this invention relates will recognize various alternative designs and embodiments for practicing the invention as defined by the following claims.



**TABLE 1**  
**Volumes (Cumulative and Per Time Interval) and Flow Rates of Human Plasma**  
**@ 28" Head Height Filtered Through 17-cm<sup>2</sup> Leukocyte-Removal Filter Devices**

Time (min)	Device M2			Device M3			Device M4			Average		Vol/time/unit area (mL/min/cm <sup>2</sup> )
	Vol	Cum Vol	Rate	Vol	Cum Vol	Rate	Vol	Cum Vol	Rate	Vol	Rate	
0.17	13.4	13.4	80.4	13.5	13.5	81.0	11.0	11.0	66.0	12.6	75.8	4.46
1	51.0	64.4	61.2	54.0	67.5	64.8	43.1	54.1	51.7	49.4	59.2	3.48
2	36.0	100.4	36.0	53.2	120.7	53.2	28.5	82.6	28.5	39.2	39.2	2.31
5	42.0	142.4	14.0	85.8	206.5	28.6	34.5	117.1	11.5	54.1	18.0	1.06
10	36.9	179.3	7.4	59.0	265.5	11.8	39.0	156.1	7.8	45.0	9.0	0.53
15	31.8	211.1	6.4	39.0	304.5	7.8	19.5	175.6	3.9	30.1	6.0	0.35
20	26.0	237.1	5.2	34.0	338.5	6.8	24.0	199.6	4.8	28.0	5.6	0.33
25	24.0	261.1	4.8									
30	24.5	285.6	4.9									

Note: Units for volume (Vol) and cumulative volume (Cum Vol) are reported in mL. Units for rate are reported in mL/min.

The average cumulative volume of human plasma delivered through the leukocyte-removal filter devices was 258.4 mL. The average volume of human plasma delivered per time per unit of EFA (mL/min/cm<sup>2</sup>), based on the results of this experiment, was 2.31 mL/min/cm<sup>2</sup>. This number represents average value at 48.3% flow decay, following delivery of 100-mL plasma after two minutes of flow.

**What Is Claimed Is:**

1. A method for purifying blood plasma which has been treated to remove erythrocytes and a substantial portion of leukocytes, an initial concentration of leukocytes remaining,  
5 comprising:

passing said blood plasma through a disposable multicomponent filter, said multicomponent filter comprising a filter stack containing one or more depth-type prefilters having a nominal pore size of from about 0.5  $\mu\text{m}$  to about 5  $\mu\text{m}$ ,  
10 at least one intermediate hydrophilic microporous membrane filter having a maximum nominal pore size of less than about 3  $\mu\text{m}$ ; and at least one final hydrophilic microporous membrane filter having a nominal pore size of from about 0.3  $\mu\text{m}$  to about 1.2  $\mu\text{m}$ , the nominal pore size of said final hydrophilic  
15 microporous membrane smaller than the nominal pore size of said intermediate hydrophilic microporous membrane, said filter stack contained in a housing, said final hydrophilic microporous membrane sealed to said housing, wherein the leukocyte concentration present in said blood plasma after  
20 passing through said filter is substantially reduced from its initial concentration.

2. The method of claim 1, wherein the leukocyte concentration is between 0 leukocytes/mL to a value which is less than about  $10^3$  of said initial leukocyte concentration.

25 3. The method of claim 1 or 2, wherein the flow rate of said disposable multicomponent filter is in excess of 1 mL/min/cm<sup>2</sup> of filter area following a filtration period of 2 minutes at a plasma head of about 70 cm or a pressure equivalent thereto.

30 4. The method of claim 1 or 2, wherein the flow rate of said disposable multicomponent filter is in excess of 2 mL/min/cm<sup>2</sup> of filter area following a filtration period of 2 minutes at a plasma head of about 70 cm or a pressure

equivalent thereto.

5. The method of claim 1, wherein said depth-type prefilter comprises a non-woven fiberglass filter having a nominal pore size of from about 0.5  $\mu\text{m}$  to about 5  $\mu\text{m}$ .

5 6. The method of claim 1, wherein at least one of said one or more intermediate hydrophilic membrane filters has a nominal pore size of from about 1.0  $\mu\text{m}$  to about 2.0  $\mu\text{m}$ .

7. The method of claim 1 or 6, wherein at least one of said one or more final hydrophilic membrane filters has a  
10 nominal pore size of from about 0.5  $\mu\text{m}$  to about 0.9  $\mu\text{m}$ .

8. The method of claim 1, wherein said filter further comprises a hydrophobic membrane, one side of said hydrophobic membrane communicating with an inside of said housing, one side of said hydrophobic membrane communicating with an  
15 outside of said housing.

9. A disposable, sterilizable filter suitable for removing substantially all residual leukocytes from blood plasma, comprising:

a housing having inlet and outlet portions thereof, said  
20 inlet portion including an inlet port, said outlet portion including an outlet port said inlet portion and outlet portion defining a flow channel therebetween;

one or more prefilters retained within said housing and fully extending across said flow channel, said prefilter(s)  
25 being positioned closer to said inlet port than said outlet port, said prefilter(s) comprising a depth-type prefilter having a nominal pore size of from about 0.5  $\mu\text{m}$  to about 5  $\mu\text{m}$ ;

at least one intermediate hydrophilic microporous membrane having a maximum nominal pore size of less than 3  $\mu\text{m}$   
30 positioned adjacent said prefilter(s) and extending across said flow channel, said intermediate hydrophilic microporous membrane(s) positioned closer to said outlet channel than said

inlet channel;

at least one final hydrophilic microporous membrane having a maximum nominal pore size of from about 0.3  $\mu\text{m}$  to about 1.2  $\mu\text{m}$  positioned adjacent said intermediate hydrophilic microporous membrane and extending across said flow channel, said final hydrophilic microporous membrane(s) positioned closer to said outlet channel than said inlet channel, said nominal pore size of at least one of said final hydrophilic membrane filters smaller than the nominal pore size of at least one of said intermediate hydrophilic membrane filters;

said final hydrophilic microporous membrane(s) being sealed within said housing such that passage of leukocytes around said final hydrophilic microporous membrane(s) is prevented; and,

wherein said inlet portion of said housing is sealed to said outlet portion of said housing.

10. A disposable, sterilizable filter suitable for removing substantially all residual leukocytes from blood plasma, comprising:

- (a) a housing having inlet and outlet portions thereof, said inlet portion including an inlet port, said outlet portion including an outlet port said inlet portion and outlet portion defining a flow channel therebetween;
- (b) one or more prefilters retained within said housing and fully extending across said flow channel, said prefilter(s) being positioned closer to said inlet port than said outlet port, said prefilter(s) comprising a depth-type prefilter having a nominal pore size of from about 0.5  $\mu\text{m}$  to about 5  $\mu\text{m}$ ;
- (c) at least one intermediate hydrophilic microporous membrane having a maximum nominal pore size of less than 3  $\mu\text{m}$  positioned adjacent said prefilter(s) and extending across said flow channel, said intermediate hydrophilic microporous membrane(s) positioned closer to said outlet channel than said

inlet channel;

(d) at least one final hydrophilic microporous membrane having a maximum nominal pore size of from about 0.3  $\mu\text{m}$  to about 1.2  $\mu\text{m}$  positioned adjacent said intermediate hydrophilic microporous membrane and extending across said flow channel, said final hydrophilic microporous membrane(s) positioned closer to said outlet channel than said inlet channel, said nominal pore size of at least one of said final hydrophilic membrane filters smaller than the nominal pore size of at least one of said intermediate hydrophilic membrane filters;

(e) means for sealing said final hydrophilic microporous membrane(s) within said housing such that passage of leukocytes around said final hydrophilic microporous membrane(s) is prevented;

(f) means for sealing said inlet portion of said housing to said outlet portion of said housing.

11. The disposable filter of claim 9 or 10, wherein at least one of said one or more depth-type prefilters comprises a non-woven fiberglass filter having a nominal pore size of from about 1  $\mu\text{m}$  to about 3  $\mu\text{m}$ .

12. The disposable filter of claim 9 or 10, wherein said filter has a flow rate in excess of about 1 mL/min/cm<sup>2</sup> of filter area following a filtration period of 2 minutes at a plasma head of about 70 cm or a pressure equivalent thereto.

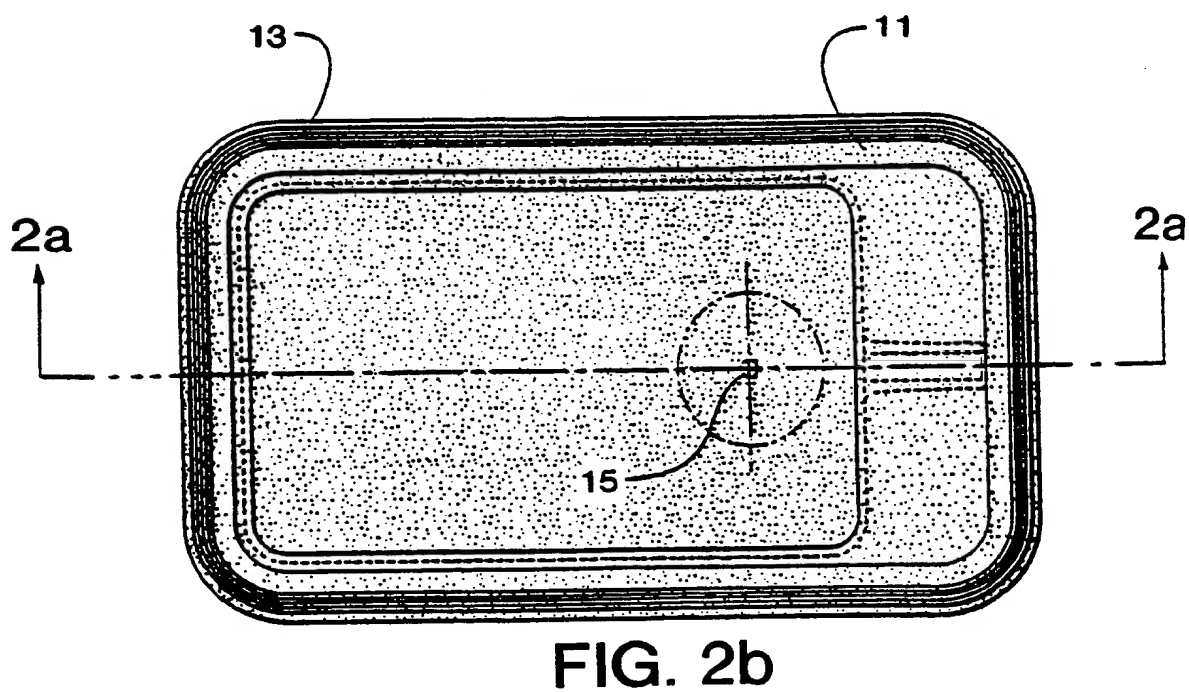
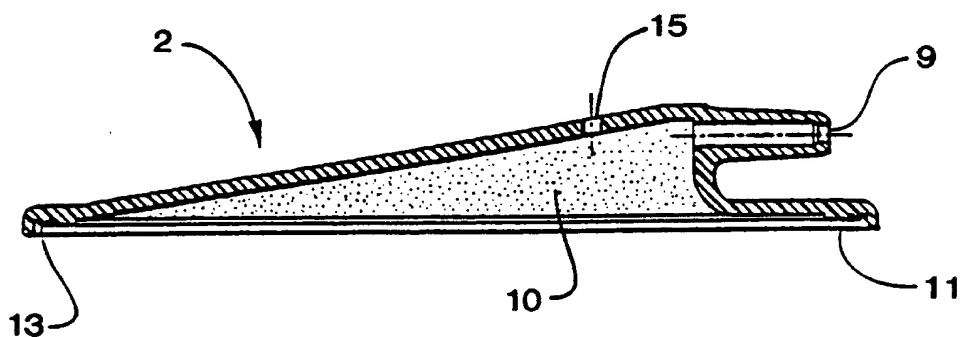
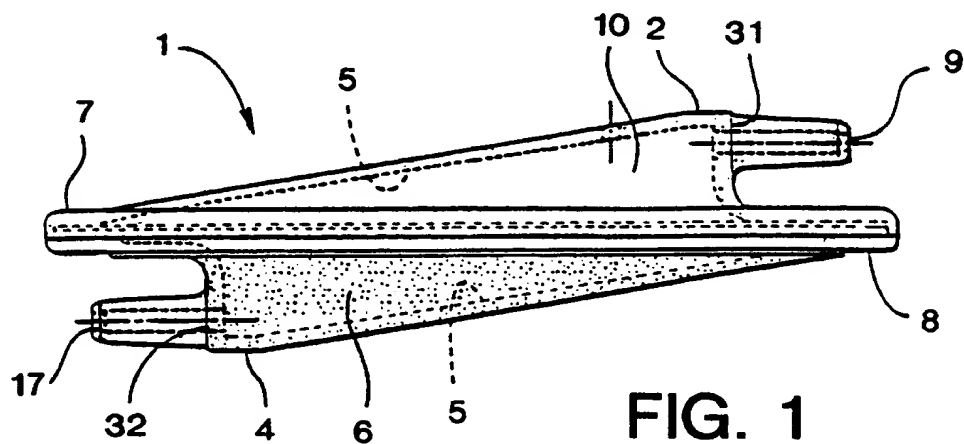
13. The disposable filter of claim 9 or 10, wherein at least one of said one or more intermediate hydrophilic membrane filters has a nominal pore size of from about 1.0  $\mu\text{m}$  to about 2.0  $\mu\text{m}$ .

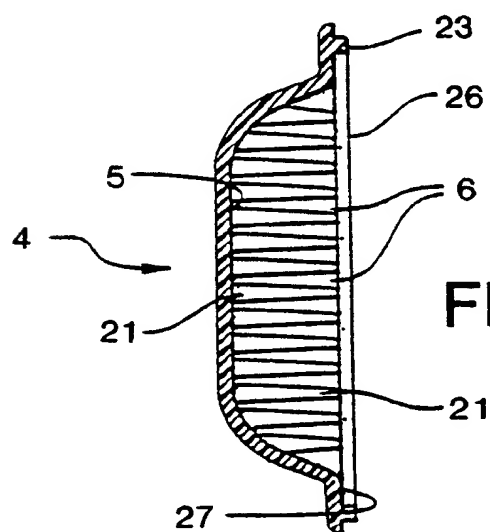
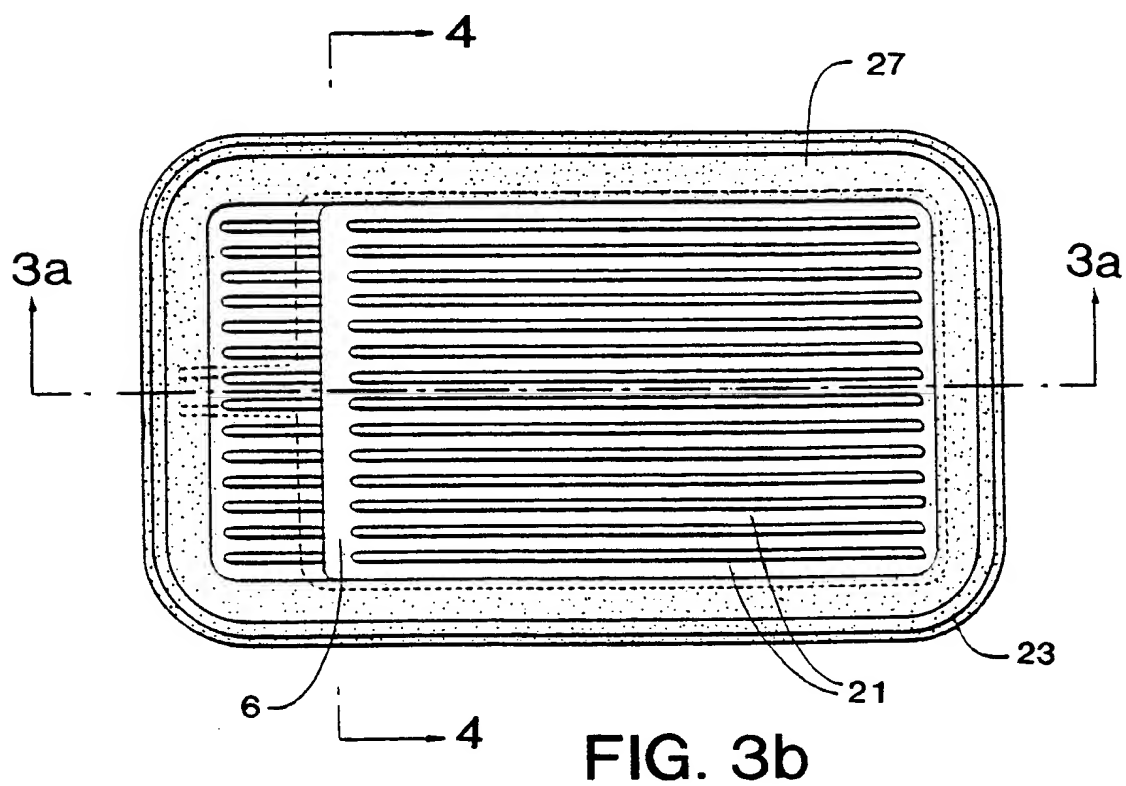
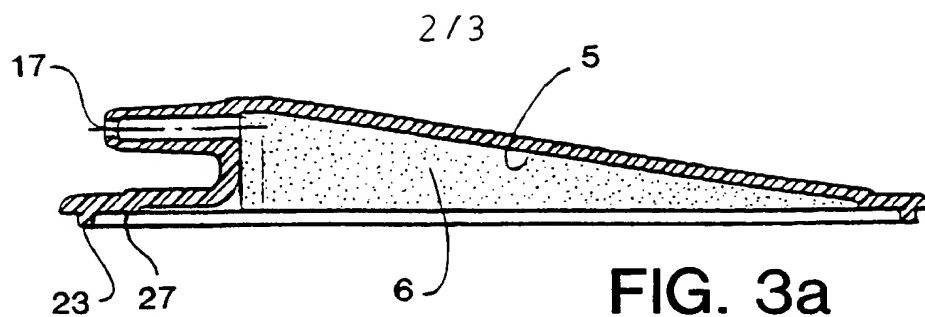
14. The disposable filter of any one of claims 9, 10 or 13, wherein at least one of said one or more final hydrophilic membrane filters has a nominal pore size of from about 0.5  $\mu\text{m}$

to about 0.9  $\mu\text{m}$ .

15. The disposable filter of claim 11 wherein said prefilter comprises fiberglass, and wherein said fiberglass when eluted with water provides an eluate with a pH of between  
5 5.5 and 8.0 and has substantially no heavy metal ions in the eluate.

1/3







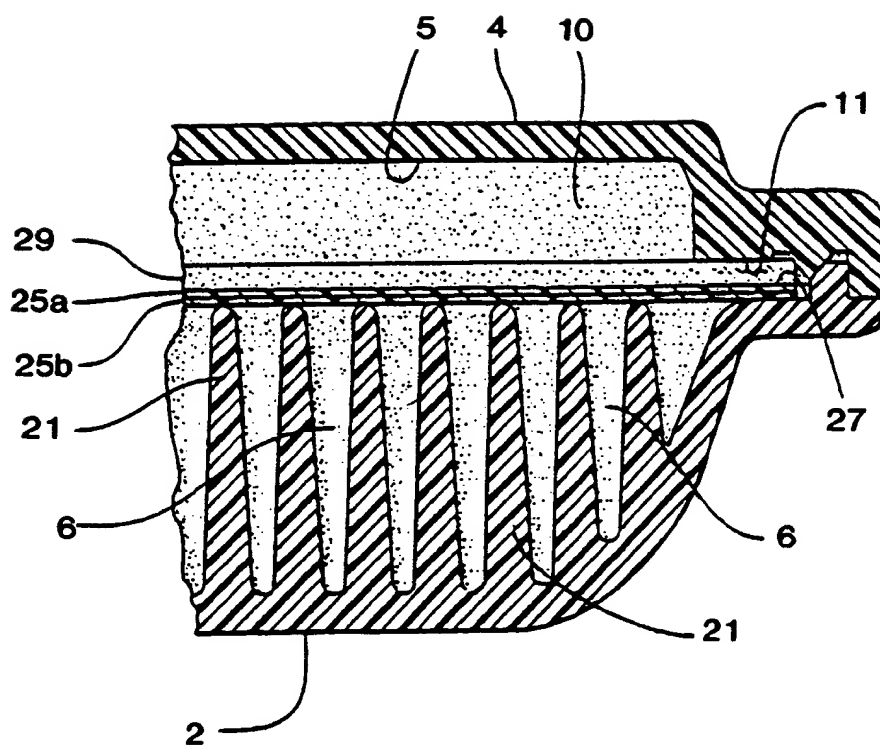


FIG. 5

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/19908

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61M1/36

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61M B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 423 989 A (ALLEN MICHAEL P ET AL) 13 June 1995 see column 2, line 29 - column 4, line 52; figure 1 ---	1,9,10
A	GB 2 037 614 A (BAXTER TRAVENOL) 16 July 1980 see page 1, line 92 - page 2, line 69; figures 1-4 ---	1,9,10
A	EP 0 554 460 A (ASAHI MEDICAL CO) 11 August 1993 see abstract -----	1,9,10



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents :

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&amp;\* document member of the same patent family

Date of the actual completion of the international search

2 March 1998

Date of mailing of the international search report

13.03.98

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Jameson, P

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/19908

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5423989 A	13-06-95	US 4987085 A	22-01-91
		US 4999287 A	12-03-91
		CA 1335783 A	06-06-95
		US 5260221 A	09-11-93
		US 5416000 A	16-05-95
		US 5264180 A	23-11-93
		US 5340539 A	23-08-94
		US 4959324 A	25-09-90
		AT 115631 T	15-12-94
		AU 626853 B	13-08-92
		AU 3384789 A	23-11-89
		DE 68919902 D	26-01-95
		DE 68919902 T	11-05-95
		EP 0342447 A	23-11-89
		JP 2138961 A	28-05-90
GB 2037614 A	16-07-80	US 5215886 A	01-06-93
		AU 5329079 A	26-06-80
		BE 880762 A	16-04-80
		BR 7908156 A	22-07-80
		CA 1127091 A	06-07-82
		DE 2948597 A	10-07-80
		DK 544879 A	22-06-80
		FI 793994 A	22-06-80
		FR 2444471 A	18-07-80
		JP 55084168 A	25-06-80
EP 0554460 A	11-08-93	SE 7910450 A	22-06-80
		CA 1127092 A	06-07-82
		JP 6024995 A	01-02-94
		WO 9303740 A	04-03-93
		US 5478470 A	26-12-95
		US 5665233 A	09-09-97
		JP 5168711 A	02-07-93

